Qualitative and quantitative investigation of glycans attached to Prostate-specific antigen (PSA) glycoprotein of healthy and cancer samples

ASMS 2013, ThP 19 339

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Introduction

Investigation of glycans of PSA
- 26 kDa protein
- Contains 237 amino acids
- N45 is glycosylated
- Used as cancer biomarker
- One sample from a healthy person and a 2nd from a cancer one
- Samples supplied in the course of the ABRF gPRG study 2013

Workflow (Figure 1)
- Bottom-up approach for identifying glycans on glycopeptides
- Intact protein approach for relative quantitation

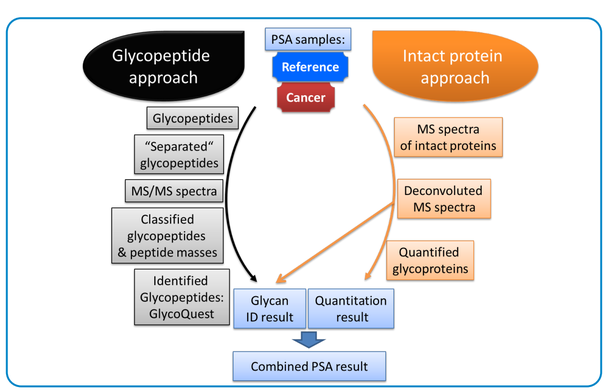

Fig. 1 Combined approach for glycan identification and relative quantitation.
Left: ID was performed on glycopeptide level. Crucial point was the classification of glycopeptide MS/MS spectra with subsequent automatic peptide mass determination and GlycoQuest database search.
Right: For quantitation, MS data of the intact protein were acquired and processed to determine the monoisotpic masses.

Literature:
[1]Stavenhagen K., Hinneburg H., Thaysen-Andersen M., Hartmann L., Varón Silva D., Fuchser J., Kaspar S., Rapp E., Seeberger P.H., Kolarich D.; Quantitative mapping of glycoprotein micro- and macro-heterogeneity: An evaluation of mass spectrometry signal strengths using synthetic peptides and glycopeptides, J.-Mass Spec. 2013, accepted.

Methods

A. Bottom-up approach

Sample preparation: ArgC digest

LC: nanoHPLC with RP column

Mass spectrometry: amaZon speed ETD (Bruker) with Captive Spray nanoBooster for CID and ETD spectra

Data processing:
- Classification in ProteinScape for detecting glycopeptide MS/MS spectra and for determination of the peptide masses
- Glycan identification using the search engine GlycoQuest, which is integrated in ProteinScape (Bruker)

B. Intact protein approach

LC: analytical HPLC with RP-4H column

Mass spectrometry: maXis 4G (QTOF, Bruker)

Data processing:
- Performed on deconvoluted (Maximum Entropy) data, monoisotopic masses were annotated using the SNAP algorithm (DataAnalysis 4.1, Bruker).
- For glycan quantitation, intensities were used.
- For glycan ID, a GlycoQuest search was performed handling the PSA protein part as a glycan modification. The MS mass tolerance was set to 15 ppm.

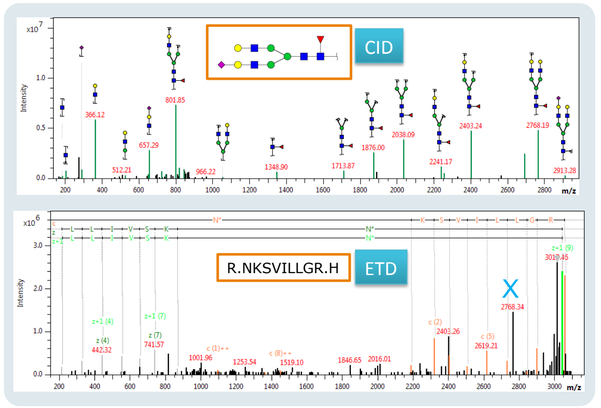

Fig. 2 Glycopeptide spectra acquired on the amaZon speed ETD for 765.59 *m/z* with charge 4+. The complete glycopeptide was identified in ProteinScape.
Top: CID spectrum for glycan ID.
Bottom: ETD spectrum for peptide ID.
The not assigned signal with m/z 2768.34 (marked with X) results from the glycopeptide precursor with one sialic acid loss.

Results

Identification

- In the bottom-up approach, CID spectra were acquired on the ion trap, and 44 glycan compositions with structure proposals were identified.
- This was confirmed by a significant score and manual inspection of the annotated spectra. An example is shown in Figure 2 (top).
- ETD spectra were also acquired on the ion trap with CaptiveSpray nanoBooster. This set-up increased the charge and improved the ETD spectra quality. This facilitated peptide ID. An example is shown in Figure 2 (bottom).
- A significant number of unspecifically cleaved (glyco) peptides was detected, resulting from the exopeptidase activity of PSA. Some glycans were identified attached to four different peptide sequences.
- In a second GlycoQuest search MS data of the intact proteins were used. This resulted in 50 glycan compositions within the admitted mass tolerance (Fig 3).

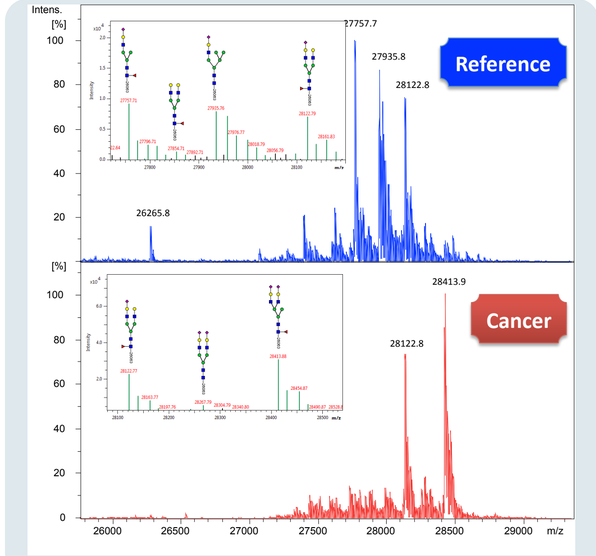

Fig. 3 MS spectra (Max. Ent. data) of the intact proteins acquired on the maXis 4G. Data processing included deconvolution and annotation of monoisotopic masses. Inserts show a zoom in the MS spectra (peaklists) after GlycoQuest search. Identified signals (in green) are labeled with glycan structure proposals.

Intact protein approach for quantitation

- Intact PSA (both samples) were measured using a high resolution QTOF instrument.
- The MS spectra show severe differences in the glycan profile (Figure 3). These alterations could be confirmed by MALDI data (data not shown).
- For the proper quantitation, intensities for all previously identified glycans were compared. The relative intensities of the most abundant glycans are listed in the table below. Glycans with increased abundance in the cancer sample are higher sialylated.

MH+ glycoprotein	Glycan composition	Relative int. cancer sample	Relative int. healthy sample
27757.6	Hex4 HexNAc3 Fuc SialA	8.8	100
27935.7	Hex6 HexNAc3 SialA	1.6	77.6
27960.7	Hex4 HexNAc4 Fuc SialA	1.6	66.8
28122.8	Hex5 HexNAc4 Fuc SialA	76.2	61.9
28138.8	Hex6 HexNAc4 SialA	33.4	19.2
28163.8	Hex4 HexNAc5 Fuc SialA	20.9	24.7
28413.9	Hex5 HexNAc4 Fuc SialA2	100	4.8
28429.9	Hex6 HexNAc4 SialA2	45.2	4.6
28454.9	Hex4 HexNAc5 Fuc SialA2	41.5	3.6

Advantages of the intact protein approach

- Since the glycans carry a huge protein residue, suppression effects known from complex glycan or glycopeptide spectra, are elimintated (1).
- No digestion artifacts compared to glycopeptide based quantitation
- Reduction of glycan charge derived artifacts

Requirement

- Only glycoproteins with a single glycosylation site can be quantified without further fragmentation of the protein.

Conclusions

Sophisticated combined workflow with:

- **Bottom-up approach** for simple, fast and reliable glycan and peptide identification
  - **Glycan ID on intact protein level** for confirming these results
- **Quantitation** on intact protein level

Glycoproteins

QITMS - ETD